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# Studies in the Behavioral Toxicology of Environmental Contaminants

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**Behavioral toxicology represents a relatively new research area in the West, and a new source of information pertinent to standard setting. Despite this abbreviated history, however, it can call on a rather advanced technology, largely provided by the rapid and extensive development of behavioral pharmacology during the past two decades. As exemplified by the U.S. contribution to the joint study of carbon disulfide, the approach derived from this background relies on the acquisition of dose–effect data with a preparation yielding stable baseline performance. The first study in this collaborative series employed pigeons trained to peck a response device consisting of a transilluminated plastic disk. Various relationships between this response and the occasions on which it led to the delivery of food were explored in order to ascertain which behavioral variables were most sensitive to acute exposures. In addition, a central nervous system drug, whose neurochemical mode of action is believed to parallel that of carbon disulfide, was tested in the same preparations. Further research on these questions is being continued with monkeys.**

Behavioral toxicology is still a young discipline in the United States. The first major conference on this topic was held at Rochester in 1972 (*1*). In the U.S.S.R., on the other hand, behavioral toxicology has occupied a central position in environmental health for many years.

Part of the reason is the immense influence of Sechenov and Pavlov on Soviet physiology. Toxicologists in the Soviet Union could hardly pass through a training program without encountering their ideas and their emphasis on the nervous system as the key system in physiology. Toxicology in the United States, in contrast, made hardly any contact with behavior because behavioral evaluations were in the tradition of experimental psychology.

Although behavioral assessment is now assuming increasing importance in the West and in the United States, it still is not as heavily emphasized as in the Soviet Union, and it comes out of a different tradition. In this country, it largely has grown out of the field of behavioral pharmacology, which experienced an explosive growth with the introduction of drugs for the control of behavior disorders. It is a field largely developed and led by psychologists and encompasses two aims. One is to develop techniques for screening new drugs, that is, preclinical psychopharmacology. The second is to unravel the behavioral mechanisms of action of drugs and drug-behavior interactions. This tradition has been carried over to behavioral toxicology, even though in toxicology the emphasis now is on discovering effects at very low levels, especially over long periods of time. This kind of problem requires a rather different perspective.

These differences in history, emphasis, and technique between the U.S. and U.S.S.R. approaches to behavioral toxicology seemed to offer a profitable source of collaboration. The avenue chosen to pursue such a collaboration was to select complementary approaches from the two countries in an attempt to jointly define the central nervous system toxicology of a specified substance and to compare the two approaches. Carbon disulfide was agreed on as the first candidate.

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The continuing interest in carbon disulfide around the world attests not only to its interesting toxicological properties but to its widespread use in a number of industrial processes. It has been a well recognized industrial hazard for decades, exposures producing a broad range of behavioral and neurological disorders. Recent evidence, furthermore, suggests that it may also produce an increased risk of death from coronary heart disease and suicide. Behavioral effects accessible to laboratory study may be particularly important as precursors or correlates of other effects, as well as significant in themselves. Carbon disulfide is particularly interesting to behavioral pharmacologists because of its neurochemical properties; it seems to act as a dopamine-$\beta$-hydroxylase inhibitor (*2*).

For our behavioral baselines, we turned to the technology of operant conditioning. Many experiments in psychopharmacology employ what is called schedule-controlled behavior as a dependent variable. A reinforcement schedule specifies a relationship between a behavioral action and its consequences. For example, a pigeon might be trained to peck a specially designed disk 150 times in order to obtain access to food for several seconds. This arrangement would be known as a fixed-ratio (FR) 150 reinforcement schedule. (The disk is usually illuminated from behind and the feeding device is a grain-filled magazine which is pulled into place by a solenoid.) Many other kinds of relationships may be arranged. For example, a fixed-interval (FI) reinforcement schedule is one on which a response is followed by the delivery of food only if a specified time has elapsed.

In earlier studies from Rochester by Armstrong and his colleagues (*3*), exposure to mercury vapor at a concentration of 17 mg/m$^3$ reduced response rate during both fixed-interval and fixed-ratio performance in pigeons. Although this represented a relatively high dose, no pathological changes ascribable to mercury were found, thus confirming the contention that mercury vapor can produce behavioral changes at concentrations that produce no pathological deficits. Such data were a reason for turning to schedule-controlled performance in pigeons as a first step in the $CS_2$ studies.

## Experiments on Carbon Disulfide

For these first experiments on $CS_2$, the test animals were 9 male white carneaux pigeons maintained at 80% ($\pm$ 5% ) of free-feeding weight. They were tested in conventional operant behavior chambers of the kind shown in Figure 1. Each front panel contained a feeding aperture, and an opening for the response key. All three chambers used for these experiments were mounted in a frame within a large, Fiberglas-insulated metal enclosure (Fig. 1).

FIGURE 1. Behavior test chambers: (top) pigeon pausing after reinforcement; (middle) pigeon pecking response key; (bottom) pigeon feeding from grain hopper during reinforcement cycle.

When the pigeon struck the key with a force exceeding 18 g, a switch behind the key closed a circuit to define a response. When reinforcement was programmed, a pivoting hopper presented mixed grain through the feeding aperture.

Three behavioral procedures were used, but only two will be described here. Both have been widely used in studies of CNS drugs, both in this and in other laboratories (*4*).

## Spaced Responding

After initial training, the pigeons assigned to this experiment were trained on a reinforcement schedule that required them to space successive responses at least 20 sec apart. Each such interresponse time resulted in reinforcement.

## Multiple Fixed-Interval Fixed-Ratio

The final parameters for this schedule were approached gradually, in small increments. The final values were fixed-interval, 10 min (FI 10); fixed ratio, 50 responses (FR 50).

FI 10 was defined as follows. In the presence of a steady white light, the first peck on the key after 10 min resulted in grain presentation. Responses emitted during the 10-min period had no specified consequence.

FR 50 was defined in this way. In the presence of a flashing white light, the 50th response on the key would produce delivery of grain. The pigeons were allowed additionally 1 min during FI 10 to complete the requirement, and 10 total min to complete the FR 50. Otherwise, the schedule components switched automatically.

## Experimental Control

As is now standard practice in this laboratory and in many others, the entire experiment was programmed and controlled by a digital computer, in this instance, a LINC.

Computers are coming to occupy an increasingly central role in behavioral pharmacology and toxicology. Aside from current economics, which make them the cheapest, most reliable control devices, they also offer a virtually unlimited flexibility, and the ability to store large amounts of data with fine temporal resolution (*5*).

In the present experiment, all three pigeon chambers were controlled simultaneously by means of a programming system devised by Gott (*6*). This system also stored relevant data about animal weights, drugs or agents and dose levels, and experimental parameters. These data, together with the data from each experimental session, were stored on magnetic tape for later processing. The primary information consisted of sequential interresponse times (IRTs), that is, intervals between pecks, recorded with a resolution of 40 msec.

## Exposures

Small Rochester exposure chambers were used to administer the $CS_2$ (Fig. 2). Each chamber consisted of a 28 × 44.5 cm glass tank mounted in a wooden frame with a 15.5 cm diameter porthole door.

The mixing flask was a modified 100-ml round-bottomed flask; one inlet was connected to the regulated air supply, the other to the $CS_2$ bubbler. A rotometer mounted on each side of the door regulated air flow, one directly into the chamber and the other through $CS_2$.

The $CS_2$ vapor was generated by placing the bubbler in an ice-water bath and bubbling air through it at a low rate which was then diluted before entering the chambers. Flow rates were adjusted to obtain a concentration of 2 mg/l.

$CS_2$ concentration was sampled approximately once per hour and read by a spectrophotometer at

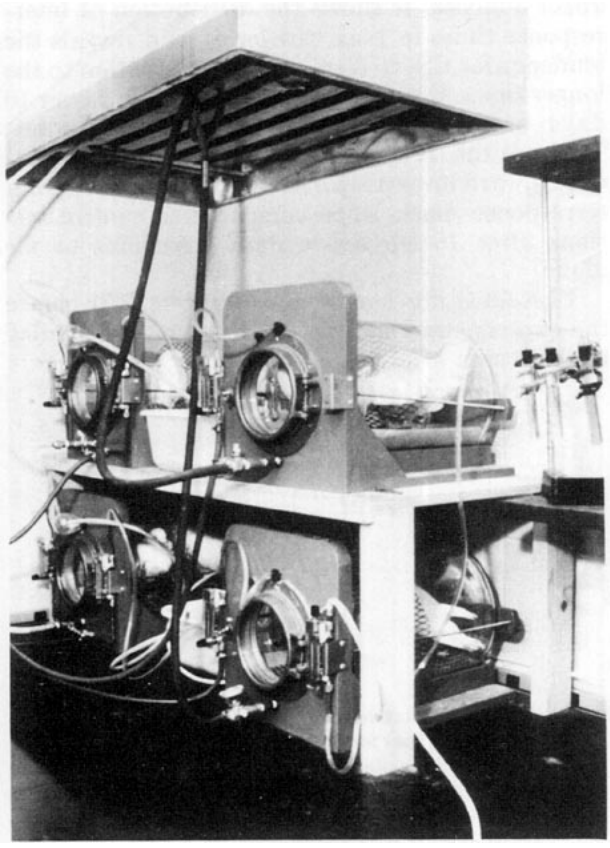

FIGURE 2. Pigeons in the exposure chambers. The two chambers on the left were used for $CS_2$ exposure. Those on the right were used for exposure to air. The test tubes on the shelf on the right are part of the air sampling apparatus.

430 nm; the absorption fluid was a modification of Viles' solution (*4*).

As a treatment comparison, bis(4-methyl-1-homopiperzinylthiocarbonyl) disulfide (FLA-63), which, like $CS_2$, is presumed to be a dopamine-$\beta$-hydroxylase inhibitor, was injected over a range of doses.

## Results

In discussing the results, we will focus on individual performance, since our aim has been to determine the applicability of operant behavior baselines to measurements of $CS_2$ toxicity in single organisms.

The spaced responding schedule, also known as differential reinforcement of low rate (DRL), proved sensitive to acute 8-hr exposures. Exposures on two consecutive days enhanced the effect. The response rate decreased, and, as a result, the number of reinforcements increased. Figure 3 gives us a direct analysis. It shows the distribution of interresponse times in 1-sec categories, and reveals the tendency for $CS_2$ to displace the distribution to the longer times. Chronic exposures of 4 hr/day over 10 days produced a similar pattern of change. Through the 10-day period, there was a gradual drift toward lower response rates, that is, longer interresponse times, when compared to control sessions after 10 successive daily exposures to air alone.

FLA-63 at doses of 40 and 80 mg/kg, 2 hr before the experimental session, produced effects similar to those seen after $CS_2$ exposure.

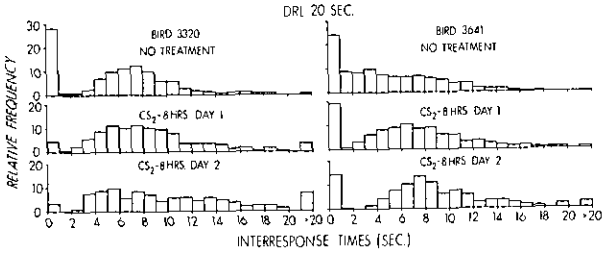


FIGURE 3. Representative relative frequency histograms of interresponse times for birds 3320 and 3641. The height of each bar represents the relative frequency, the frequency of IRTs of a given duration as a per cent of all IRTs in the behavioral session. The interresponse time is inversely related to response rate, i.e., for control session shown, bird 3320 has a mean response rate of 0.13 responses/sec and mean IRT of 7.7 sec; bird 3641 has a mean response rate of 0.21 responses/sec and a mean IRT of 4.8 sec.

The three pigeons maintained on the multiple FI 10-FR 50 schedule proved similar in their response to $CS_2$. Figure 4 shows cumulative records for bird 629. Such records are produced by an upward increment of the recording pen for each response, with the paper moving at a uniform speed. Each tracing shows, first, the FI component, then (except for the bottom tracing), the pen deflection that marks reinforcement, then the 50-response fixed ratio, then, finally, the reinforcement marker that terminates this component. Of course, many such samples constituted a single experimental session.

The first 8-hr exposure produced a severe decline in and disruption of FI responding, but left FR performance nearly intact. After another day of $CS_2$ exposure, FI performance, usually characterized by a preponderance of responses near the end of the interval, was even more disrupted than on the previous day. FR performance also showed evidence of disruption, but less severe—a frequent

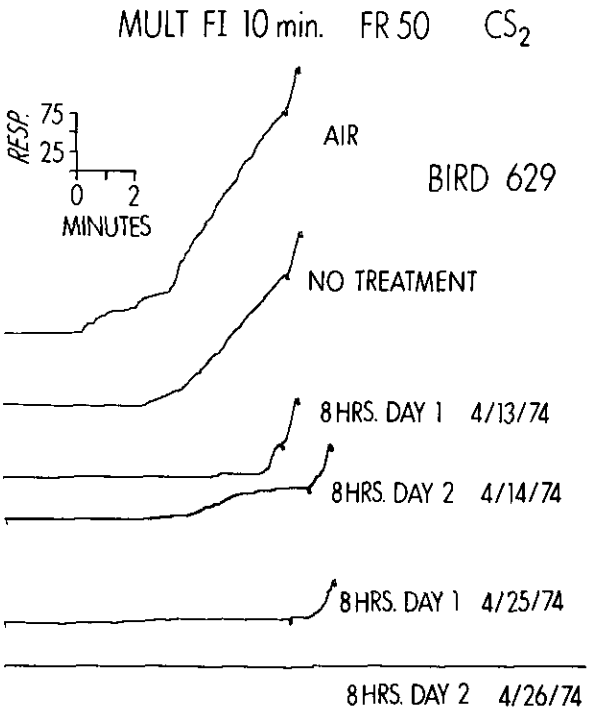


FIGURE 4. Representative cumulative records for bird 629, working on a multiple fixed interval fixed ratio schedule of reinforcement. One fixed interval and the succeeding fixed ratio was taken from the middle of the behavioral session. The top two records show two control sessions. The lower records indicate various exposure to $CS_2$. In the bottom record, there was no responding; the upward pen movements indicate switching of the schedule components.

finding in behavioral pharmacology. Subsequent exposures produced markedly exaggerated effects.

Similar patterns of effects were seen after 40 mg/kg of FLA-63.

This first study on $CS_2$, with pigeon subjects, indicates that a variety of behaviors are sensitive to its effects. The most sensitive appear to be those that tend to occur at low or moderate rates (FI, DRL). Those that occur at high rates (FR) seem to be less sensitive. Perhaps other schedules should be devised to more carefully explore this point.

The correspondence between the actions of $CS_2$ and FLA-63 seemed rather close. It is tempting to attribute this correspondence to their common ability to act as inhibitors of dopamine-$\beta$-hydroxylase. Whether this mechanism is the means by which classical $CS_2$ intoxication is produced cannot be stated now. The neurochemical data are ambiguous (*4*). It is certainly a question worth pursuing, however.

## Future Directions

Both the U.S. and U.S.S.R. participants are planning to extend their findings to other situations. One aspect of the U.S. plan is the use of nonhuman primates, particularly under conditions of chronic exposure.

Further extensions of the cooperative program should place more emphasis on coordination of methods. One approach would be for both participants to compare brain electrical activity, and to use similar behavioral situations. It is hoped that the plan to use testing equipment from the Rochester laboratory in Moscow will make it possible to approximate the latter aim.

An additional set of problems needs to be examined also. Certain substances may cause behavioral effects by indirect actions on the central nervous system, a point stressed by Soviet toxicologists. Respiratory irritants, for example, may make it difficult for a subject to perform a complex task at a consistently high level. Noxious smells may not produce physiological damage, but bear a cost just the same in the aversive environment they produce. A joint program to develop new methods for such problems would be a useful contribution to environmental health.

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